Conclusion: Herein c-Myc acts as a key master regulator of in vitro migration, invasion and radioresistance. In fact, c-Myc depletion alone seems to be sufficient to block the in vitro pro-metastatic abilities and to radiosensitize ERMS cells. In addition, our data suggest c-Myc as important, but not essential, in controlling the molecular machinery responsible for cancer neo-angiogenesis. In conclusion these data strongly suggest that the targeting of c-Myc can be tested as a promising strategy for an anti-cancer therapy.

EP-2063

Apoptotic pathway activation in prostate neoplastic cells after 12 Gy-IORT

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Purpose or Objective: To evaluate apoptotic pathways involved in prostate cancer treated with intraoperative radiotherapy (IORT) with 12 Gy, studying the effects on cancer cells, prostatic intraepithelial neoplasia (PIN) and normal cells

Material and Methods: Since 2005, 111 patients treated at University Hospital of Novara, Italy with local advanced prostate adenocarcinoma were treated with radical prostatectomy and 12 Gy IORT followed by 50 Gy postoperative radiotherapy. In this setting, we selected a sample of 10 patients for a preliminary feasibility study. Selection criteria for this phase were: no neoadjuvant hormone therapy, Gleason score > 7. Proteins involved in the apoptotic cascade (Bax, Caspases -3 and -9) were studied before and after 12 Gy single shoot in neoplastic cells, high grade PIN areas and in normal prostate cells. Immunofluorescent detection of antigens (anti-Bax, anti-caspases-3 and -9), were performed on bioptic sample and on surgical specimens 5-mm slices. On surgical specimens there were also detected Bcl-2, and ki-67 with immunoistochimical analysis. A count of positive spots for immunofluorescence (Bax+, Caspases-3 and -9+/all nuclei, 40x magnification) was performed on tumor cells, PIN, healthy tissue areas. Bax and caspases immunofluorescent positivity was compared in different areas and in neoplastic areas before and after single shoot high dose

Results: A significant increase in Bax, Caspases-3 and -9 expression was detected in tumor and PIN areas comparing IORT treated and untreated samples ($p<0.05$). After 12 Gy-single dose, healthy areas expressed significantly lower level of Bax and caspases positive with respect to neoplastic cells ($p<0.0001$), while in PIN areas, Bax positive cells were significantly more present than in neoplastic areas ($p=0.0001$). Mean Bcl-2 in neoplastic cells is 17% (range: 1-23), mean ki-67 in neoplastic area is 4.5% (range: 1-17). With multivariate analysis, we find that cancer cells with Ki-67 ≥ 8% show a trend toward greater expression of Bax ($p=0.0641$)

Conclusion: After 12 Gy irradiation, Bax and caspases resulted overexpressed in tumor and PIN cells, in particular in prostate cancer with higher proliferation index. PIN areas seem to be more radiosensitive than neoplastic areas and healthy cells do not activate apoptosis after single shoot, showing an intrinsic radioresistence. This preliminary study represents the basis for an extensive work in which we would correlated clinical parameters with pathology and apoptotic factors. In fact, the comprehension of these relationships could allow to better understand the mechanisms of high dose per fraction and, radioresistence in order to personalize treatments

EP-2064

Radiation induces metabolic switch to lactate production to support tumour cell survival

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Purpose or Objective: Purpose: Radiation treatment of tumor cells resulted in a reduction of endogenous ATP levels. Aim of this study was to elucidate the molecular scenario standing behind this observation.

Material and Methods: Endogenous ATP-levels were dermined by ATP-ELISA. HIF1a, PDK1, LDH and PDH expressions were visualized by western blotting. Lactate production was quantified by lactate-assay. Cellular survival was proved by clonogenic survival assay.

Results: Results: Ionizing radiation induced expression of Hif1 alpha even at clinical relevant doses of 2 Gy. Hif1alpha induced activation of mitochondrial PDK1, which results in PDK1 dependent phosphorylation of pyruvate dehydrogenase (PDH). PDH is responsible for conversion of pyruvate to acetyl-CoA, which fuels the TCA cycle. Thus, irradiation blocks TCA cycle and mitochondrial activity. Simultaneously Hif1alpha induced expression and activity of lactate dehydrogenase (LDHA) to convert glucose to lactate. Indeed we observed a clear increase in lactate production in tumor cell lines in response to irradiation. Furthermore, inhibition of PDH activity was associated with mitophagy and ATP-depletion, which explains the radiation induced ATP drop down. In addition, this radiogenic switch to lactate production reduced production of mitochondrial derived radicals and increased cellular radio-resistance. Pretreatment with the Hif1 alpha inhibitor BAY87-2243 prevented the radiogenic switch to lactate metabolism and radio-sensitized the tumor cells. In addition, tumor cells are strictly dependent from high glucose supply after irradiation and can be radio-sensitized by blockage of radiogenic glucose uptake with glucose transporter SGLT inhibitor Phlorizin.

Conclusion: In summary, we could show, that tumor cells switch in a Hif1 alpha dependent manner to anaerobe glucose metabolism to generate ATP, which renders cells radio-resistant. Blockage of Hif1 alpha stabilization or blockage of glucose uptake radio-sensitized tumor cells.

EP-2065

Effects of spontaneous γH2AX level on radiation-induced response in human somatic cells

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Purpose or Objective: Phosphorylated histone H2AX (γH2AX) foci are well-known markers of DNA double-strand breaks in human cells. Spontaneous γH2AX foci form on unrepaired DNA double strand breaks, shortened telomeres and sites with altered chromatin conformation. The presence of such permanent γH2AX foci in cell is an important component of epigenetic background and potentially lead to the activation of DNA repair system. The objective of this study was to analyze the effects of spontaneous γH2AX level on radiation-induced response in human somatic cells.

Material and Methods: Spontaneous γH2AX foci and radiation-induced micronuclei were analyzed in peripheral blood lymphocytes of 54 healthy individuals after exposure to 2 Gy ionizing radiation in vitro. Further, a transcriptome analysis was performed using gene expression microarrays in lymphocytes of two sub-groups of individuals: 1)